PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 37/54	A1	(11) International Publication Number: WO 91/06313
		(43) International Publication Date: 16 May 1991 (16.05.91)

(21) International Application Number: PCT/US90/06086

(22) International Filing Date: 26 October 1990 (26.10.90)

(30) Priority data:
427,879 27 October 1989 (27.10.89) US

(71) Applicant: GENENCOR INTERNATIONAL INC. [US/US]; 180 Kimball Way, South San Francisco, CA 94080 (US).

(71)(72) Applicants and Inventors: KOLATTUKUDY, Pappachan, E. [US/US]; 2301 Hoxton Court, Columbus, OH 43220 (US). O'NEILL, Roger, A. [US/US]; 3180 Melendy, San Carlos, CA 94070 (US). POULOSE, Ayrookaran, J. [IN/US]; 2540 Carmel Drive, San Bruno, CA 94066 (US).

(74) Agent: DILLAHUNTY, T., Gene; Burns, Doane, Swecker & Mathis, P.O. Box 1404, Alexandria, VA 22313-1404 (US).

(81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.

(54) Title: PROTECTION OF GROWING CROPS FROM PATHOGENS BY TREATMENT WITH ENZYMES

(57) Abstract

A method is disclosed for protecting a growing crop from pathogens comprising applying to the growing crop and to any pathogen present thereon a compound capable of generating an active oligomer elicitor in the crop or the pathogen. The compound is applied with a penetrating agent which aids in the entry of the compound into the cuticle layer of the growing crop or the pathogen. The elicitor is generated in the crop or the pathogen in an amount effective to elicit production in the crop of an anti-pathogenic agent. When the elicitor is generated in a pathogen present on the harvested crop, the elicitor is generated in sufficient amount to transfer into the crop, which transfer may be aided by the presence of said penetrating agent, in order to produce the desired phytoalexins in the harvested crop. Also disclosed is a composition comprising the above compound and a penetrating agent for the compound as well as a growing crop treated with the above composition.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MR	Mauritania
BE	Belgium	GA	Gabon	MW	Malawi
BF	Burkina Faso	GB	United Kingdom	NL	Netherlands
BG	Bulgaria	GR	Greece	NO	Norway
BJ	Benin	HU	Hungary	PL	Poland
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	SD	Sudan
CF	Central African Republic	KP	Democratic People's Republic of Korea	SE	Sweden
CG	Congo			SN	Senegal
CH	Switzerland	KR	Republic of Korea	SU	Soviet Union
CI	Côte d'Ivoire	LI	Liechtenstein	TD	Chad
CM	Cameroon	LK	Sri Lanka	TG	Togo
DE	Germany	LU	Luxembourg	US	United States of America
DK	Denmark	MC	Monaco		

PROTECTION OF GROWING CROPS FROM PATHOGENS BY TREATMENT WITH ENZYMES

FIELD OF THE INVENTION

The present invention relates to protection of crops and, more particularly, to methods and compositions for protecting crops from pathogens utilizing each crop's own natural defense system.

BACKGROUND OF THE INVENTION

Over the years various techniques have been developed for protecting growing crops from attacks by various pathogens. Generally, the approach taken has been to apply to the crops various synthetic and naturally derived fungicides, bactericides and anti-viral agents (anti-pathogenic agents) which protect the crop from being infected without otherwise deleteriously affecting the growth and ultimate harvesting of the crop. While many such compositions are effective, there has nonetheless been a growing concern among consumers in the recent past as to the potential harmful side effects of chemical anti-pathogenic agents. This, in turn, has led to an increasing interest among both food producers and food vendors for compositions of natural origin, which are far less likely to cause adverse side effects and which would be more acceptable to the growing number of concerned consumers.

As discussed by Abersheim et al., "Oligosaccharins", Scientific American, volume 253(3) September 1985, plants themselves are known to produce compounds which increase their resistance to pathogens. More specifically, plants are now known to contain regulatory molecules called oligosaccharins which,

when in active form, appear to deliver a message regulating a particular plant function including defense against disease as well as regulation of morphogenetic pathways. Such oligosaccharins are fragments of the cell wall which are released from the cell wall by enzymes, there being different oligosaccharins released by different enzymes. Once released, the oligosaccharins, which appear to be highly specific, are recognized by the plant and stimulate plant tissue to synthesize antibiotics. The enzymes which cause the release of the oligosaccharins from the cell wall can originate from an invading organism such as a fungus, bacterium or virus or from the cells of the plant itself, such as when such cells have been damaged.

For example, as reported by Davis et al. in "Host-Pathogen Interactions", Plant Physiology, Volume 74, pp. 52-60 (1984), it is known that plants, when invaded by potentially pathogenic microorganisms, can accumulate at the site of infection phytoalexins which are antimicrobial compounds of low molecular weight. Such phytoalexin accumulation is in fact induced by oligosaccharins of microbial origin called elicitors. Compounds which cause release of such oligosaccharin elicitors from cell walls that have been isolated include fungal cell wall glucans and several fungal glycoproteins including a fungal endopolygalacturonase. Elicitors, e.g., pectic oligogalacturonides, have also been released from soybean cell walls by acid hydrolysis. Similar oligogalacturonide elicitors solubilized from cell walls, called endogenous elicitors, have been obtained by partial acid hydrolysis of the walls of suspension-cultured tobacco, sycamore, and wheat cells and from citrus pectin.

It is further known that heat-labile elicitors can be produced by E. caratovora grown on a defined medium containing citrus pectin. These elicitors were released using the enzymes α-1,4-endopolygalacturonic acid lyases (PGA), which are pectin-degrading enzymes that have been shown to be secreted by many plant pathogens. Evidence is also present to the effect that release of oligosaccharides from the pectic polymers of plant cell walls by the PGA triggers the elicitation of phytoalexin accumulation. This evidence further suggests that the release of endogenous elicitors from plant cell walls by pectin-degrading enzymes plays a role in general plant disease resistance to microorganisms.

It has further been reported by Davis et al, "Host Pathogen Interactions XXXI" Plant Molecular Biology, Vol. 6, pp. 23-32 (1986) that phytoalexin accumulation can be induced in vitro by abiotic and biotic elicitors. Abiotic elicitors include detergents and heavy metal salts, such as $HgCl_2$. Biotic elicitors include a variety of compounds isolated from microorganisms and plant tissues. It was found that, in the induction of phytoalexin accumulation in soybean cotyledons, a hexa-β-glucosyl glucitol elicitor acts synergistically with either the deca-α-1,4-D-galacturonide elicitor or PGA lyase, an enzyme that releases the decagalacturonide from pectic polysaccharides. Dilute organic-acid buffers were also found to enhance the elicitor activity of the hexa-β-glucosyl glucitols.

It has also been shown that oligosaccharides produced from fungal cell walls can elicit phytoalexin and chitinase accumulation. Hadwiger, L.A., and

Beckman, J.M. "Chitosan as a Component of Pea Fusarium Solani Interactions" Plant Physiol. 66; 205-211 (1980); Kendra, D.F. and Hadwiger, L.A. "Characterization of the Smallest Chitosan Oligomer that is Maximally Antifungal to Fusarium Solani and Elicits Pisatin Formation in Pisum Sativum" Exp. Mycol., 8: 276-281 (1984); Roby, D., Gadelle, A., and Toppan, A. "Chitin Oligosaccharides as Elicitors of Chitinase Activity in Melon Plants" Biochem. Biopys. Res. Comm. 143: 885-892 (1987). The active oligosaccharides include those of chitin, a β-1,4-linked polymer of N-acetyl glucosamine, and chitosan, a closely related material composed of β-1,4-linked glucosamine residues. Both the phytoalexins and chitinases elicited by these materials can be antifungal. The β-glucan elicitors, which are among the most potent biotic elicitors, have been characterized by Sharp, J., Valent, B., and Albersheim, P. "Purification and Partial Characterization of a β-Glucan Fragment That Elicits Phytoalexin Accumulation in Soybean" J. Biol. Chem. 259: 11312-11320 (1984). (See related Sharp et al.: J. Biol. Chem. 259: 11321-11326 and 11341-11345).

While the above-described natural defenses elicited on growing crops by the presence of pathogenic agents is theoretically interesting, it is nonetheless a practical reality that, by the time the cell material of a growing crop produces the much needed anti-pathogenic agent, the growth of the pathogen has often advanced to the point where such anti-pathogenic agents are of little effect.

While oligosaccharin elicitors have been applied to plants in an attempt to "trick" a plant into producing anti-pathogenic agents, i.e., by contacting such plants with oligosaccharins, such attempts have, with the

exception of application of elicitors at wound sites, been unsuccessful thereby leading one to conclude that it is not merely the presence of the elicitors generated by pathogens which elicits the anti-pathogenic response by the plant. Thus, while theoretically interesting, there has thus far been little practical application of the above-described plant protection mechanisms since the time period between the initial elicitation of anti-pathogenic agents by a pathogen and the actual production of meaningful quantities of such anti-pathogenic agent is often too long for such anti-pathogenic agents to be effective.

SUMMARY AND OBJECTS OF THE INVENTION

In view of the foregoing limitations and shortcomings of the prior art methods of protecting growing crops from pathogens, as well as other disadvantages not specifically mentioned above, it is apparent that there still exists a need in the art for a method and composition for protecting a growing crop from pathogens which does not require the application thereto of potentially harmful chemicals. It is, therefore, a primary objective of the present invention to fulfill that need by providing a technique for protecting a growing crop from pathogens which makes use of anti-pathogenic agents derived from the crop itself. More particularly, it is an object of the present invention to provide techniques, compositions and methods for protecting a growing crop from pathogens by the application to that crop of a compound capable of causing anti-pathogenic agents to be produced by the crop itself before a pathogen causes the production of the anti-pathogenic agents, thereby protecting the crop better from attack or damage from the pathogen.

It is a further object of the present invention to provide a technique for protecting a growing crop from pathogens wherein the anti-pathogenic agents produced by the growing crop are elicited independently of the pathogens themselves thereby eliminating the unduly long lag times between attack by a pathogen and production of an effective amount of anti-pathogenic agent.

It is a further object of the present invention to provide a technique for protecting a growing crop from pathogens wherein the anti-pathogenic agents produced by the growing crop are elicited by materials released from the cell walls of pathogens present on the crop prior to a time that those materials would normally be released from the pathogens present. Thus, the early release of the materials from the pathogens cause the crop to produce anti-pathogenic agents earlier than would normally occur, thereby reducing or eliminating the lag time between when the pathogen may attack the crop and the production of an effective amount of anti-pathogenic agents by the crop to protect itself from the pathogen.

In a first aspect, the present invention comprises a method for protecting a growing crop from pathogens comprising applying to the crop a compound capable of generating an elicitor which stimulates the crop to synthesize anti-pathogenic agents, the compound being derived from a source other than a pathogen growing on the crop and the elicitor being generated in situ from the crop or from the pathogen tissue in an amount effective to elicit production of an anti-pathogenic agent by the crop.

In one embodiment of the first aspect, the invention comprises a method for protecting a growing crop from pathogens wherein the compound capable of generating an elicitor, such as chitinase, chitosanase or endo β-glucanase, is applied to the crop to generate active oligosaccharides, such as β-glucans, chitosan and chitin oligosaccharides, from cell walls of an invading pathogen present on the crop, the oligosaccharides contacting the plant to produce a localized anti-pathogenic response.

In one aspect, the present invention provides a method for protecting a growing crop from pathogens comprising applying to said growing crop a composition comprising:

(a) a compound capable of generating in said crop or in a pathogen present on said crop an elicitor which stimulates said crop to synthesize anti-pathogenic agents, and

(b) a penetrating agent capable of assisting said compound in penetrating into the cuticle layer of said crop or pathogen;

said compound comprising an endoenzyme derived from a source other than a pathogen growing on said crop and applied to said crop in an amount effective to cause said elicitor to be generated <u>in situ</u> in an amount effective to elicit production of an anti-pathogenic agent by the crop.

In another aspect, the present invention relates to a composition for application to a growing crop to protect said growing crop from pathogens comprising:

(a) a compound capable of generating in said crop or in a pathogen present on said crop an elicitor which stimulates said crop to synthesize anti-pathogenic agents;

(b) a penetrating agent capable of assisting said compound in penetrating into the cuticle layer of said crop;

said compound comprising an endoenzyme derived from a source other than a pathogen growing on said crop and present in an amount effective to cause said elicitor to be generated in situ in an amount effective to elicit production of an anti-pathogenic agent by the crop.

In another aspect, the present invention relates to a growing crop protected from pathogenic attack comprising said crop having a composition on a surface of said crop comprising:

(a) a compound capable of generating in said crop or in a pathogen present on said crop an elicitor which stimulates said crop to synthesize anti-pathogenic agents, and

(b) a penetrating agent capable of assisting said compound in penetrating into the cuticle layer of said crop or pathogen;

said compound comprising an endoenzyme derived from a source other than a pathogen growing on said crop and present in an amount effective to cause said elicitor to be generated in situ in an amount effective to elicit production of an anti-pathogenic agent by the crop.

With the foregoing and other objects, advantages, and features of the invention that will become hereinafter apparent, the nature of the invention may be more clearly understood by reference to the following detailed description of the invention and to the appended claims.

DESCRIPTION OF PREFERRED EMBODIMENTS

The "compounds capable of generating an elicitor" which are applied to the harvested crop according to this invention, are typically endoenzymes and similar compounds which cause the crop or an invading pathogen to release active oligomer elicitors, such as oligosaccharins, oligogalacturonides, β-glucans, chitins, chitosan, etc., from the cell walls of the crop or the pathogens present on the crop. The endo- compounds are useful in the present invention because, it is believed, they produce active oligomers from the crop or pathogen tissue or cell materials, which oligomers are active as elicitors.

The compounds effective against pathogens which are elicited by the elicitors and produced by the crop are referred to as "anti-pathogenic agents."

The compounds capable of generating elicitors in crops can be derived from fungi, bacteria, viruses, or from the harvested crop itself. When the crop involves a food product, the compounds must be recognized as being safe for application to fields and, of course, for application to food products. Included among such compounds are endoenzymes such as endocarbohydrases or endolyases which produce active oligomers by breaking up polysaccharide chains at various points along the length of the chain (as opposed to exoenzymes which act only at the end of the chain). Among the endoenzymes, endoglycanases are preferred such as endopolygalacturonase and pectate lyase, pectin lyase, β-glucanase, chitinase, and chitosanase. Particularly preferred are the endoglycanases. Also, especially preferred are endopolygalacturonases such as those prepared from the fungi Fusarium monniliforme and Aspergillus niger.

The "penetrating agent" component of the composition of this invention can be any material which causes or assists said elicitor-generating compound to enter the cuticle layer of the growing crop or a pathogen present on the growing crop. In those compositions wherein said compound is an enzyme which is itself capable of entering or passing through the cuticle layer of the crop, the penetrating agent used will be a surfactant, a humectant, or other material which aids in the efficient contact of said compound with the cuticle layer and causes the entry of said compound into the cuticle layer to be faster or more efficacious. In those compositions wherein said compound is not itself capable of penetrating the cuticle layer in a reasonable manner or in a reasonable amount of time, the penetrating agent used usually will be a cutinase, wax esterase, or exoenzyme which is capable of entering or penetrating the cuticle layer faster or more efficiently than said compound and which will aid or speed the entry of said compound into the cuticle layer of the crop or the pathogen. In some cases it may be desirable to use such a penetrating agent in order to preserve the activity of said compound so that it will still be capable of generating the desired elicitors once it has entered the cuticle layer of the crop. When the penetrating agent is an enzyme, the composition can also comprise other useful components such as a surfactant, humectant or the like to further enhance the efficiency of the practice of this invention. As will be recognized the penetrating agent may also function to aid the entry of an elicitor into the cuticle layer of the growing crop where the elicitor is generated by said compound in a pathogen present on the growing crop and the elicitor then transfers from the pathogen to the growing crop

thereby causing production of phytoalexins in the crop. Appropriate penetrating agents may also be selected from the materials known in the art as "adjuvants" for agricultural chemicals, for example from the adjuvants disclosed in copending application Serial No. 07/112,108, filed October 19, 1987, incorporated herein by reference.

The compositions of this invention will typically contain additional components known to be useful for applying materials to growing crops, such as one or more carrier materials, buffer materials for pH control, and the like. Water will often be used as a carrier, but other conventional carriers may also be used.

It should be understood that when a composition and method of this invention are employed, the composition is applied to the growing crop and to any pathogen which may be present on the surface of the growing crop. The composition of this invention can generate an elicitor from the crop or from the pathogen, whereby the elicitor enters the crop and causes production of anti-pathogenic agents. Thus, the compounds for use in this invention can be selected and formulated to generate the elicitors desired for particular anti-pathogenic agent production. In some cases it will be desirable to generate the elicitor or elicitors in the crop itself in order to cause certain anti-pathogenic agents to be produced to protect the crop from pathogen attack. In other cases it will be desirable to generate the elicitor in the pathogen present on the growing crop and cause that elicitor to enter the crop in order to cause production of specific anti-pathogenic agents to protect the crop against attack from that particular pathogen. This can be timed to

cause the crop to produce its anti-pathogenic agents well in advance of when the crop would have normally been induced to do so by the actual attack of the crop by the pathogen. Thus, by the time the pathogen is mature enough or reaches its life cycle point where it attacks the crop, the crop is already protected against the attack, whereas normally the crop is not stimulated to produce the anti-pathogenic agents until attacked by the pathogen and then cannot produce those agents rapidly enough to protect itself.

In general, the compositions of this invention are applied to the growing crops in accordance with techniques well known to persons skilled in the art such as in the form of a spray prepared by mixing the enzyme compound and penetrating agent into a suitable carrier such as a buffer solution. A buffer is preferably employed in view of the fact that enzymes are typically quite pH sensitive and thus, are desirably protected from potentially damaging variations in pH. It will be appreciated, however, that it is possible that the natural pH of a crop being treated, which is typically acidic, will be within the acceptable pH range of the enzyme being applied thereto. In such instances, a buffer may not be required. A surfactant is usually employed in order to wet the surface of the crop to thereby obtain exposure of the crop to the enzyme. Typically, the amount of active enzyme compound in the total composition including the carrier is between about 1 and about 1000 μg/ml, although amounts outside such range might also be acceptable, depending upon the compound being employed and the crop being treated. The amount of the surfactant required is readily ascertainable by persons skilled in the art and will typically range from between about 0.001 and about 0.1 % by weight based on

the weight of the carrier. It should be noted that, while the compositions of this invention are primarily described as intended for use on growing crops, the compositions of this invention will also find utility on harvested crops as well. Treatment of harvested crops is the subject of copending application Serial No. _____, filed on even date with this application (Attorney Docket No. 010055-011).

Where pH regulation is not important, such as where the natural pH of the crop corresponds to the pH at which the applied compound is active, water may serve as the carrier. Where a buffer is employed, there may be used any buffer solution which is compatible with the enzyme and does not otherwise deleteriously affect the crop. Of course, the buffer must also be a material which is known to be safely used with a foodstuff. Suitable buffers include, for example, a 5-50 mM solution of sodium succinate or sodium citrate. As the surfactant, there can be employed, once again, compounds which are compatible with the enzymes being applied, do not otherwise deleteriously affect the crop being treated and are safely used with a foodstuff. Suitable surfactants include, but are not limited to Tween-80 and Triton-X-100.

The above-described formulations, containing the compound capable of generating an elicitor in the crop or in a pathogen present on the crop, the penetrating agent, and, if desired, a surfactant in a suitable carrier are then applied to the growing crop. Application of the formulation can be carried out in accordance with techniques well known to persons skilled in the art such as by preparing a spray for application to the growing crop. It will be appreciated that the amount of solution required for

treatment of the crop will depend on the particular crop being treated. Such amount is one which is readily ascertained by a person skilled in the art.

It is noted that the formulations of the invention can prevent pathogenic attack, e.g., fungal, bacterial, or viral attack on a crop. This is accomplished as mentioned above by causing the crop to produce anti-pathogenic agents in advance of actual attack upon the crop by a pathogen, so that when the pathogenic attack does occur, the plant is prepared, either by an immune response or otherwise.

Upon application of the formulations of the invention to a growing crop, elicitors are generated in the crop or in a pathogen present on the crop. It is noted that in addition to oligosaccharins such as oligogalacturonides, the compounds of the invention might generate other elicitors present in the cellular material of the crop. Furthermore, such elicitors can elicit production of a number of anti-pathogenic agents including, but not limited to, phytoalexins, chitinase, beta-1,3-glucanase and various proteinase inhibitors.

The following are examples of preferred embodiments of the compositions of this invention.

Example I

Endopolygalctuonase	20 µg/ml
Buffer	10 mM sodium phosphate pH 7.0
Cutinase	20 µg/ml
Triton X-100	0.05% wt/vol

Example II

Pectin lyase	20 µg/ml
Cutinase	20 µg/ml
Buffer	10mM sodium phosphate 7.0
Triton X-100	0.05% wt/vol

Example III

Pectate lyase	20 µg/ml
Cutinase	20 µg/ml
Buffer	10mM sodium phosphate pH 7.0
Triton X-100	0.05% wt/vol

Example IV

B-glucanase	50 µg/ml
Cutinase	20 µg/ml
Buffer	10mM sodium phosphate pH 7.0
Triton X-100	0.05% wt/vol

Example V

Chitinase	50 µg/ml
Cutinase	20 µg/ml
Buffer	10mM sodium phosphate 7.0
Triton X-100	0.05% wt/vol

Example VI

Chitosanase	50 µg/ml
Cutinase	20 µg/ml
Buffer	10mM sodium phosphate 7.0
Triton X-100	0.05% wt/vol

Example VII

Pectin lyase	50 µg/ml
Buffer	10mM sodium succinate pH 5.2
Triton X-100	0.1% wt/vol

Example VIII

Endopolgalaturonase	50 µg/ml
Buffer	10mM sodium succinate pH 5.2
Triton X-100	0.1% wt/vol

Example IX

Pectate lyase	50 μg/ml
β-glucanase	50 μg/ml
Chitinase	50 μg/ml
Cutinase	20 μg/ml
Triton X-100	0.05% wt/vol
Buffer	10mM sodium phosphate pH 7.0

In a preferred aspect of the method of this invention, it is desired to spray the crop before or at the first sign of the presence of pathogen or of the infection of the crop by pathogen. A desired application rate is at 40 gal/acre. It may be desired in many crops to repeat the treatment after two weeks if necessary to proavide the desired protection from the pathogens present on the crop.

The following is a list of crops and corresponding diseases and organisms which can be controlled in accordance with the present invention.

CROP	DISEASE	ORGANISM
CORN	Anthracnose	Colletotrichum graminicola
	Smut	Ustilago maydis
	Ear and Stalk rot	Gibberella roseum
	Leaf-sot	Helminthosporium carbonum
COTTON	Blight	Ascochyta gossypii
	Fusarium Wilt	Fusarium oxysporum vasinfectum
	Southern blight	Solerotium rolfsii
	Verticillium Wilt	Verticillium albo-atrum
FIELD BEANS	Anthracnose	Collectotrichum lindemuthianum
	Root rot	Fusarium solani phaseoli
	Rust	Rust Uromyces phasoli
		Paeudomonas syringae pv phaseolicola

CROP	DISEASE	ORGANISM
FLAX	Anthracnose	Colletotrichum lini
	Stem break	Polyspora lini
	Wilt	Fusarium oxysporum f. sp. lini
OATS	Anthracnose	Collectotrichum graminicola
	Rust	Puccinia coronate var. avenae
	Darkstem	Septoria avenae
PEANUT	Leaf spot	Cercospora arachidicola
	Stem rot	Solerotium rolfsii
MUSTARD	Black spot	Alternaria brassicae
RAPESEED	Black leg	Leptospheria maculans
RICE	Bakanae	Fusarium moniliforme f. sp.
	Blast	Pyricularia oryzae
	Stem rot	Sclerotium oryzae
	Leaf spot	Rhizoctonia oryzae
	Sheath spot	
SAFFLOWER	Wilt	Fusarium oxysporum f. sp. carthami
SORGHUM	Anthracnose	Colletotrichum graminicola
	Stalk rot	Fusarium moniliforme
	Root rot	
SOYBEANS	Aerial blight	Rhizoctonia solani
	Anthracnose	Colletotrichum dematium
	Wilt	Fusarium oxysporum vasinfectum and glycines
	Late Blight	Phytophtora megasperma glycinea
	stem canker	Diaporthe phaseolorum
		Pseudomonas syringae pv glycinea
SUNFLOWER	Black stem	Phoma oleracea
	Wilt	Verticillium dahliae

CROP	DISEASE	ORGANISM
WHEAT	Anthracnose	Colletotrichum graminicola
	Bunt	Tilletia caries T. foetida
	Stem rust	Puccinia graminis tritici
	Head blight	Fusarium roseum gerealis
POTATO	Late Blight	Phytophthora infestans
ALFALFA		Stemphylium botryosum
BROAD BEAN		Botrytis fabae
SWEET POTATO		Ceratocystis fimbriata
TOMATO		Cladosporium fulvum Pseudomonas syringae pv. tomato

Although only preferred embodiments of the invention are specifically illustrated and described above, it will be appreciated that many modifications and variations of the present invention are possible in light of the above teachings and within the purview of the appended claims without departing from the spirit and intended scope of the invention. It will also be apparent that many pathogenic diseases not listed may be effectively treated or prevented by employing the methods and compositions of this invention.

WHAT IS CLAIMED IS:

1. A method for protecting a growing crop from pathogens comprising applying to said growing crop a composition comprising:

(a) a compound capable of generating in said crop or in a pathogen present on said crop an elicitor which stimulates said crop to synthesize anti-pathogenic agents, and

(b) a penetrating agent capable of assisting said compound in penetrating into the cuticle layer of said crop or pathogen;

said compound comprising an endoenzyme derived from a source other than a pathogen growing on said crop and applied to said crop in an amount effective to cause said elicitor to be generated in situ in an amount effective to elicit production of an anti-pathogenic agent by the crop.

2. The method of Claim 1 wherein said endoenzyme comprises endopolygalacturonase, pectate lyase, pectin lyase, β-glucanase, chitinase, or chitosanase.

3. The method of Claim 1 wherein said penetrating agent comprises a surfactant, a humectant, an exoenzyme, a cutinase or a wax esterase.

4. The method of Claim 1 wherein said elicitor produced is an oligosaccharin.

5. The method of Claim 1 wherein said composition comprises a buffer.

6. The method of Claim 1 wherein said composition comprises from about 10 to about 1000 µg/ml of said compound.

7. The method of Claim 1 wherein said endoenzyme comprises an endoglycanase.

8. The method of Claim 1 wherein said endoenzyme comprises an endocarbohydrase or endolyase.

9. The method of Claim 1 wherein said endoenzyme comprises an endopolygalacturonase.

10. The method of Claim 1 wherein said compound applied to said crop generates an active oligosaccharide from cell walls of an invading pathogen present on said crop, said oligosaccharide contacting the said crop to produce an anti-pathogenic response in said crop.

11. The method of Claim 10 wherein said active oligosaccharide is chitosan or chitin oligosaccharide.

12. A composition for application to a crop to protect said crop from pathogens comprising:

(a) a compound capable of generating in said crop or in a pathogen present on said crop an elicitor which stimulates said crop to synthesize anti-pathogenic agents;

(b) a penetrating agent capable of assisting said compound in penetrating into the cuticle layer of said crop;

said compound comprising an endoenzyme derived from a source other than a pathogen growing on said crop and present in an amount effective to cause said elicitor to be generated in situ in an amount effective to elicit production of an anti-pathogenic agent by the crop.

13. The composition of Claim 16 wherein said endoenzyme comprises endopolygalacturonase, pectate lyase, pectin lyase, β-glucanase, chitinase, or chitosanase.

14. The composition of Claim 12 wherein said penetrating agent comprises a surfactant, a humectant, a cutinase or a wax esterase.

15. The composition of Claim 12 wherein said elicitor produced is an oligosaccharin.

16. The composition of Claim 12 wherein said composition comprises a buffer.

17. The composition of Claim 12 wherein said composition comprises 10-1000 μg/ml of said compound.

18. The composition of Claim 12 wherein said endoenzyme comprises an endoglycanase.

19. The composition of Claim 12 wherein said endoenzyme comprises an endocarbohydrase or endolyase.

20. The composition of Claim 12 wherein said endoenzyme comprises an endopolygalacturonase.

21. The composition of Claim 12 wherein said compound applied to said crop generates an active oligosaccharide from cell walls of an invading pathogen present on said crop, said oligosaccharide contacting the said crop to produce an anti-pathogenic response in said crop.

22. The composition of Claim 21 wherein said active oligosaccharide is chitosan or chitin oligosaccharide.

23. The composition of Claim 12 comprising a carrier.

24. The composition of Claim 16 comprising a carrier.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/06086

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A 61K 37/54

U.S. Cl: 424/94.61

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.Cl	424/94.61; 435/72, 71/65; 536/20;435/178,84

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

BIOSIS: ELICITOR, CUTICLE, PLANT, ENZYM?,PERMEABIL?

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	US, A, 4,762,547 (IWASAKI et al.) 09 August 1988. See col. 1, lines 37 to 42.	1-37
Y,P	US, A, 4,891,096 (AKKAWI) 02 January 1990. See col. 2, lines 28-37.	1-37
Y	US, A, 3,911,110 (SMIRNOFF) 07 October 1975, See col. 1, lines 27-32.	1-37

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
18 December 1990	08 FEB 1991
International Searching Authority [1]	**Signature of Authorized Officer** [20]
ISA/US	Jane Williams

Form PCT/ISA/210 (second sheet) (May 1986)